

NASA TTF-9330

FORMATION OF MELANOIDINES FROM CHITIN

S. M. Manskaya, T. V. Drozdova, and K. I. Tobelko

FACILITY FORM 502	N65-22621	
	(ACCESSION NUMBER)	(THRU)
	12	1
	(PAGES)	(CODE)
		06
	(NASA CR OR TMX OR AD NUMBER)	(CATEGORY)

Translation of "Obrazovaniye Melanoidinov
iz Khitina". Doklady Akademii Nauk SSSR,
Vol. 96, No. 3, pp. 569-572, 1954.

GPO PRICE \$ _____

OTS PRICE(S) \$ _____

Hard copy (HC) \$ 1.00

Microfiche (MF) \$.50

FORMATION OF MELANOIDINES FROM CHITIN

S.M. Manskaya, T.V. Drozodova, and K.I. Tobelko
(Presented by Academician A.P. Vinogradov, January 13, 1954)

BIOCHEMISTRY

ABSTRACT

22621

Chitin is a high-molecular compound found extensively in nature. Recently, bacteria which destroy chitin have been discovered. Melanoidines result from the interaction of amino acids and sugar (Maillard reaction).

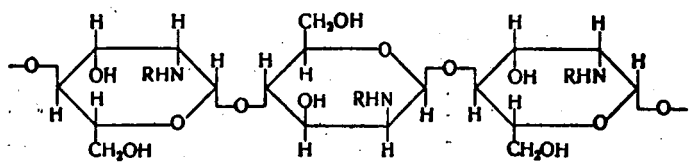
The authors try to simulate the process of melanoidine formation from glucosamine and chitin. The process is found to be similar to the melanoidine formation process from sugar and amino acid. Using the Debye powder pattern method, the authors find melanoidines to be amorphous, whereas chitin and glucosamine are crystalline.

Author

Chitin is a natural, high-molecular compound which belongs to /569* the hydrocarbon group. Chitin is found extensively in nature; it is included in the composition of shells belonging to different arthropodae

*Note: Numbers in the margin indicate pagination in the original text.

(crayfish, crabs, and hexapods). The cellular walls of fungi, certain types of algae (Ref. 1), and bacteria are composed of chitin. Chitin is insoluble in water and organic solvents, and it is immune to the action of alkalis. During hydrolysis with acids, chitin yields equivalent amounts of glucosamine and acetic acid. Therefore, it can be assumed that acetoglucosamines underline the structure of chitin. The structure of chitin can be schematically shown by the following formula:

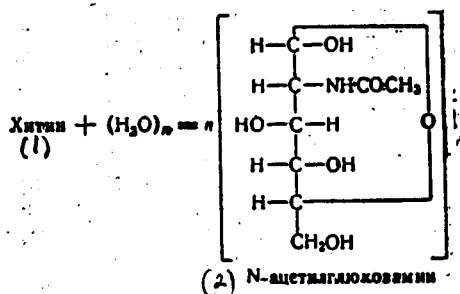


The majority of monographs point to the immunity of chitin to disintegration by bacteria (Ref. 2, Ref. 3). Chitin, which was obtained from the fossil remains of insect wings located in lignite, was found to be unchanged. This was studied by Abderhalden and Heyns (Ref. 4), and is usually used as an example for the stability of chitin. According to information given by A. P. Vinogradov (Ref. 5), chitin-type mantles belong to the oldest ones, and the remains of chitin, shells and skeletons have been found, beginning with the late Proterozoic and Paleozoic.

In spite of the relative stability of chitin and its extensive abundance, there is no information about where it might be accumulated in large amounts in mineral rocks, the earth, or in marine deposits. In recent years, many bacteria have been discovered which destroy chitin. F. I. Kopp and Ye. M. Markianovich (Ref. 6) have shown the wide abundance of chitin-destroying bacteria in marine deposits, in sediment and in the sea water, on the basis of their own data and on the basis of data given

in the literature. Prior to this, B. L. Isachenko (Ref. 7) characterized the biological decay of chitin in the soil, due to the effect of the chitinase enzyme of bacteria and different fungi.

Chitin is decomposed by the chitinase enzyme, with the formation of N-acetylglucosamine (Ref. 8).



(1) - Chitin; (2) - N-acetylglucosamine

The decomposition product of chitin is glucosamine, which includes the glucose and amine group, and it merits a great deal of attention. It is well known from the data given in the literature that amino acids/570 and sugar freely react when heated, with the formation of products which have a dark color and which are called melanoidines. This reaction, the so-called Maillard reaction (Ref. 9), has been studied for several processes, both natural and technological. V. L. Kretovich and P. P. Tokareva (Ref. 10), who studied the formation of melanoidines in mixtures of different sugars and amino acids, assumed that this reaction is related to oxidizing-reducing conversions, which take place most readily with pentoses, and is accompanied by the reduction of amino acid in the experimental mixture and the accumulation of aldehydes. The presence of melanoidines produces the coloration and aroma during the preparation of red rye malt and in bread baking (Ref. 11). A. M. Kuzin and O. I. Polyakova

(Ref. 12) assume that in the interaction of sugars and amino acids, the intermediate substance is N-glucoside. During this reaction, they separated out the calcium salt of N-glucoside. This opinion was confirmed by later studies (Ref. 13, Ref. 14). The Maillard reaction was repeated (Ref. 13, Ref. 15) under moderate conditions (pH 7 and $T = 37^{\circ}$), which was particularly important for its study in natural processes. There are many studies which are devoted to the participation of melanoidines in the formation of humic acids (Ref. 16, Ref. 17).

TABLE 1
CHANGE IN THE INTENSITY OF COLORING
(In mg J_2 per 1 ml of solution)

(3) Пробы	Продолжительность опыта в часах (1)							
	(2) до нагрев.	1	3	6	8	11	14	20
(4) Глюкозамин	0,0	0,6	0,6	1	1,6	2	20	20
(5) Глюкозамин + гликокол	0,0	2	2	4	40	40	>200	>200

(1) Experiment Duration in Hours; (2) Before Heating; (3) Samples;
(4) Glucosamine; (5) Glucosamine + Glycocol

In spite of the numerous studies on humic acids (Ref. 18), the origin of the nitrogen-containing component of humic acids, as well as the nitrogen in coal (Ref. 19), has not been fully clarified. The participation of chitin decomposition products in the natural processes of humate formation and the formation of coal has not been discussed up to the present time. This is probably due to set opinions about the biological stability of chitin.

We have based our study on the chemical structure of chitin, and also on the possibility of its biological decomposition into glucosamine. We have assumed that, under natural conditions in soils and in marine sediments, chitin decomposes by means of the chitinase enzyme into glucosamine, and the latter is converted into melanoidines.

We have primarily tried to reproduce the process by which melanoidines are formed from free glucosamine and chitin. Well-purified preparations of chitin were prepared from fungi (*Boletus edulis* and *B. scaber*) and from shells of river crayfish (*Potamobius astacus*), based on the method given by N. I. Proskuryakov (Ref. 20). In addition, a preparation of glucosamine was prepared from the shells of river crayfish based on the method given by Ye. Fisher (Ref. 21).

TABLE 2
CHANGE IN THE CONTENT OF AMINO NITROGEN
(In mg N in the reaction mixture)

(3) Пробы	(1) Продолжительность опыта в часах			
	(2) начало опыта	20	24	100
(4) Глюкозамин	67,5	50	38,15 выпал осадок	33,15
(5) Глюкозамин + гликокол	101,1	46,1 выпал осадок	44,6	19,17

- (1) Duration of Experiment in Hours; (2) Beginning of Experiment;
(3) Samples; (4) Glucosamine; (5) Glucosamine + Glycocol; (6) Deposit Precipitated; (7) Deposit Precipitated

The following samples were used for the experiment: (1) 1 g chitin /571 mixed in 15 ml distilled water; (2) 1 g chitin and 0.25 g glycocol in

15 ml distilled water; (3) 1 g glucosamine dissolved in 15 ml distilled water; (4) 1 g glucosamine and 0.25 g glycocoll in 15 ml distilled water.

The pH of the mixture in all samples was established at 7 - 7.5. All the samples were maintained at 93 - 95 degrees and a constant volume was maintained.

Glucosamine solutions began to turn dark after one hour of heating, and the addition of glycocoll considerably increased the intensity of the color. At the beginning of the experiment, the solutions were clear, colorless, then light yellow, brick red, and almost black by the end of the experiments. After 20 - 24 hours of heating, a fine, dark brown deposit began to separate out, the amount of which increased with further heating.

We made the following determinations during the course of the experiment: (1) establishment of the color intensity as compared with standard iodine solutions; (2) determination of amino nitrogen, according to the Van-Slayku method; (3) determination of furfural by the colorimetric method (Ref. 22) and oxymethylfurfural by the weight method with phloroglucinol (Ref. 10). The results which were obtained are set forth in Tables 1 - 3.

These data show that the process by which melanoidines are formed from glucosamine is similar to the process by which they are formed from sugars and amino acids. It is also accompanied by a darkening of the solutions, a reduction of the amino nitrogen, and accumulation of aldehydes.

In samples with chitin, after being heated for 15 - 20 hours, the

TABLE 3

CONTENT OF FURFURAL AND OXYMETHYLFURFURAL

(In mg in the reaction mixture)

(3) Пробы	(1) Фурфурол	(2) Оксиметил-фурфурол
(4) Глюкозамин . . .	1,15	Следы (6)
(5) Глюкозамин + Гли- кокол	1,0	2,5

(1) Furfural; (2) Oxymethylfurfural; (3) Samples;

(4) Glucosamine; (5) Glucosamine + Glycocoll; (6) Traces

solution took on a light yellow color; after 100 hours - a reddish brown color. Insoluble chitin gradually turns brown, and amino nitrogen appeared in the solution. The addition of glycocoll accelerated the process.

The deposit, which was precipitated in samples with glucosamine and glucosamine + glycocoll, was separated in a centrifuge, washed with water, and dried with alcohol and ether. The preparation which was obtained represented a powder which had a dark brown color. The preparation was insoluble in water, insoluble in ethylalcohol and butylalcohol, ether, acetone, benzene, ethylacetate, chloroform, dioxane, and formate. It was insoluble in alkalis (when heated, it dissolved /572 to an insignificant extent). It was insoluble in hydrochloric acid. In 10% nitric acid, it was partly soluble in the cold, and when heated it was fully dissolved.

Table 4 shows the elementary composition of a melanoidine preparation, which was obtained from glucosamine, as compared with the elementary composition of glucosamine and the elementary composition of melanoidines from sugars and amino acids.

TABLE 4

(1) Препараты	C, %	H, %	N, %	O, %
(2) Солянокислый глюкозамин	33,43	6,50	6,50	37,12
(3) Меланоидины из глюкозамина + гликокол	52,80	5,78	18,96	22,46
(4) Меланоидины из глюкозамина	52,51	4,99	4,5	38,0
(5) Меланоидины из сахаров и аминокислот*:				
(6) по Эндерсу и Тейс	54,73	5,17	5,04	35,08
(7) по Амблеру	60,0	5,0	3,5	31,15
(8) по Рукдексель	59,5	4,9	5,3	34,3
(9) по Майяру	59,1	6,0	4,6	31,3

- (1) Preparations; (2) Glucosamine Hydrochloride; (3) Melanoidines from glucosamine + glycoll; (4) Melanoidines from glucosamine; (5) Melanoidines from sugars and amino acids*; (6) According to Enders and Teys; (7) According to Ambler; (8) According to Rukdekel'; (9) According to Maillard

* Borrowed from Enders (Ref. 16).

The properties which have been described and the elementary composition of melanoidines, prepared from glucosamine and glucosamine with glycoll, point to the fact that melanoidines represent a significantly condensed nitrogen-containing compound.

The condensed nature of melanoidines is confirmed by an X-ray diffraction study (see Figure 1 which is inset on page 581, and Table 5). The Debye powder patterns show that chitin and glucosamine are crystalline compounds, and melanoidines are amorphous compounds.

Additional research is required on the chemical composition of melanoidines from glucosamine and chitin, and also the process by which melanoidines are formed under natural conditions must be studied. We shall continue our work in this direction.

TABLE 5

INTERPLANAR DISTANCES d AND LINE INTENSITIES I FOR FIGURE 1.

THE ESTIMATE OF LINE INTENSITY IS VISUAL.

№ п. п.	d , Å	I	№№ п. п.	d , Å	I
(1) Хитин			(2) Глюкозамин		
1	3,45	4	1	5,18	3
2	3,47	10	2	4,97	3
3	3,13	7	3	3,85	10
4	2,88	5	4	3,67	9
5	2,61	8	5	3,48	9
6	2,32	8	6	3,30	9
7	2,16	4	7	3,13	4
8	2,10	3	8	2,81	8
9	1,97	4	9	2,60	7
10	1,89	4	10	2,48	5
11	1,73	2	11	2,31	5
			12	2,23	7
			13	2,13	5
			14	1,99	6
			15	1,32	4

(1) Chitin; (2) Glucosamine

V. I. Vernadskiy Institute of Geochemistry
and Analytical Chemistry
USSR Academy of Sciences

Received
January 13, 1954

REFERENCES

1. Frey, R., Ber. d Schweiz. Bot. Ges., 60, 199, 1950.
2. P. P. Shorygin. Chemistry of Hydrocarbons (Khimiya uglevodov), p. 244, 1932.
3. Korshak, V. V. Chemistry of High-Molecular Compounds (Khimiya vysokomolekulyarnykh soedineniy). p. 450, Izd. AN SSSR, 1950.
4. Abderhalden, E., Neyns, K. Biochem. Zs., 259, H. 4-6, 320, 1933.
5. Vinogradov, A. P. Elementary Chemical Composition of Sea Organisms (Khimicheskiy elementarnyy sostav organizmov morya). Part III, p. 196, 1944.

6. Kopp, F. I., Markianovich, Ye. M. DAN (Doklady Akademii Nauk SSSR), 75, No. 6, 1950.
7. Isachenko, B. L. Periroda, No. 2, 97, 1939.
8. Samner, B., Somers, G. Chemistry of Enzymes and Methods for Investigating them (Khimiya fermentov i metody ikh issledovaniya). IL (Foreign Literature Publishing House), p. 127, 1948.
9. Maillard, L. S. C. R., 156, 1159, 1913.
10. Kretovich, V. L., Tokareva, R. R. Biokhimiya, 13, No. 6, 508, 1948.
11. Kretovich, V. L., R. R. Tokareva, and others. Proceedings of the N-Research Institute on the Bread Baking Industry. (Tr. N.-issl. inst. khlebopekarn. prom.), No. IV, 134, 1951.
12. Kuzin, A. M., Polyakova, O. I. Biokhimiya, 6, No. 2, 113, 1941.
13. Gottschalk, A., Partridge, S. M. Nature, 165, No. 4200, 684, 1950.
14. Taufel, K., Iwainsky, H. Biochem. Zs., 323, No. 4, 299, 1952.
15. Lea, C. H., Hanan, R. S. Nature, 165, No. 4194, 438, 1950.
16. Enders, C. Koll. Zs., 85, H. 1, 289, 1938; Biochem. Zs., 3/2, H. 5-6, 339, 1942.
17. Kononova, M. M. The Problem of the Soil Humus and Contemporary Problems Encountered in Studying it (Problema pochvennogo gumusa i sovremennyye zadachi ego izucheniya), p. 55, 1951.
18. Kononova, M. M. Pochvovedeniye, No. 12, 1953.
19. Kirner, V. In the Collection: Chemistry of Solid Fuels (Sborn. Khimiya tverdogo topliva). 1, p. 102, 1951.
20. Proskuryakov, N. I. Biochem. Zs., 167, H. 1-3, 68, 1926.

21. Guben, I. Metody Organicheskoy Khimii, 3, No. 1, 354, 1934.
22. Belozerskiy, A. N., Proskuryakov, N. I. Practical Handbook on Plant Biochemistry (Prakticheskoye rukovodstvo po biokhimii rasteniy), p. 24, 1951.

Scientific Translation Service
1144 Descanso Drive
La Canada, California